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Annual Report

For the Period January 1, 1952 to December 31, 1952

ONR Project N6 onr-26414

Cornell University

FLOW BIREFRINGENCE STUDIES IN SOLUTIONS OF MACROMOLECULES

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INTRODUCTION

This report summarizes the work done on this project during the period January 1, 1952 to December 31, 1952. The references cited herein pertain to the Bibliography at the end of the report. The Bibliography also includes papers which were published this year but described in previous reports.

RESULTS AND DISCUSSION

1. Equilibria in the fibrinogen-fibrin conversion (the activation step)

Having cited evidence (1) for the reversibility of the thrombin-induced fibrinogen-fibrin conversion, work has been directed toward the determination of the thermodynamic parameters characterizing the equilibria involved in this process. The clotting process may be thought of in terms of three reversible reactions:

(a) the thrombin-induced activation of fibrinogen, a process in which a low molecular weight polypeptide is split out from the fibrinogen molecule.

(b) the reaction between activated fibrinogen molecules to form a series of intermediate, elongated polymers.

(c) the cross-linking of these polymers to form the fibrin gel network.

Most of the attention during the past year has been devoted to steps (a) and (b). These steps will continue to occupy our attention in the coming year.

Considering first step (a), the attempt is being made to determine the equilibrium constant and its dependence on temperature. To do this, conditions must be found under which only this step proceeds. Therefore, an extensive investigation of thrombin-fibrinogen mixtures in various media has been carried out by means of flow birefringence measurements to determine, among other things, conditions where the polymerization steps (b and c) are inhibited, without denaturing the proteins. Several systems have proven to be suitable and equilibrium studies are being carried out in them (5, 9). Preliminary results indicate that the peptide bonds broken in the liberation of the polypeptide are much stronger than those usually found in simple peptides. Further work is proceeding along these lines on the basis of several hypotheses to account for such strong peptide bonds in a protein.

2. Equilibria in the fibrinogen-fibrin conversion (the partial polymerization step)

Considering next step (b), flow birefringence studies have been carried out to characterize the distribution of intermediate polymers formed when the reaction is allowed to proceed at various pH values (3, 6).

Measurements were made on thrombin-fibrinogen mixtures at various times from the moment of mixing up to the gel point at each pH. In some cases, hexamethylene glycol was used to stop the reaction at various stages. Sedimentation velocity measurements were made in conjunction with the flow birefringence measurements to aid in the interpretation of the data.

The distribution of polymers of activated fibrinogen below pH 6 appear to be of relatively low molecular weight in contrast to the high molecular weight ones observed between pH 6 and 10. These polymers (at any pH) are believed to be in equilibrium with each other and with the monomer (thrombin-activated fibrinogen). These phenomena are thought to arise from the same electrostatic effects suggested by Shulman and Ferry (J. Phys. and Colloid Chem., 54, 66 (1950) to explain the dependence of clotting time on pH in similar systems.

3. Hydrodynamic properties of proteins

In last year's report (submitted January 15, 1952) we pointed out a new approach to the consideration of the hydrodynamic properties of porteins (4). Among other things, it was shown that it was necessary to re-examine the basis for regarding chain-unfolding as an important process in protein denaturation. In the case of horse serum albumin, it was pointed out that existing data in the literature could be interpreted in terms of swelling of the molecule rather than chain-unfolding.

Work has been started to examine the situation (from the new point of view) for the denaturation of other proteins. The denaturation of fibrinogen by urea has been chosen as a first example for study (8). Flow birefringence and viscosity results obtained so far suggest that the molecule may split in half in urea, with a subsequent swelling of the split portions. Further hydrodynamic measurements are in progress on this problem. Also we are starting osmotic pressure investigations to settle the question of the molecular weight in this multi-component system

(fibrinogen + urea + buffer + water) in order to confirm the postulated splitting of the molecule.

4. Methylation of bovine serum albumin

This work, carried out in collaboration with Dr. H. A. Saroff of the National Institutes of Health, is now essentially completed. By means of hydrodynamic and infra-red measurements it has been possible to identify the structural changes produced during the methylation of bovine serum albumin (7). In the case of one of the reagents used, dry acid methyl alcohol, it has been found possible not only to identify the process as an esterification of carboxyl groups but also to obtain quantitative correlation between the optical densities in the infra-red absorption spectra and the number of methoxyl groups introduced, as determined by analysis. Structural features of the modified albumins have been examined from the point of view of their hydrodynamic properties.

Plans for the Coming Year

Most of our efforts (besides those indicated above) will be directed towards the further investigation of the equilibria involved in the fibrinogen-fibrin conversion, and also towards extensive investigations of the hydrodynamic properties of native and denatured proteins in light of our new approach. As a result of recent progress and developments in connection with the project research we plan to expand our efforts along these lines. To aid in this expansion, we have acquired, as a very useful adjunct to our flow birefringence apparatus, a Spinco ultracentrifuge;

also it is hoped that funds will soon be available from the National Science Foundation for a post-doctoral fellow who, in addition to our present personnel, could carry out some of our proposed investigations of hydrodynamic properties of protein solutions. Some of the systems on which we plan to work in the coming year include, besides urea-denatured fibrinogen, modified bovine serum albumin, heat denatured ovalbumin, gelatin and derivatives of other natural products such as nitrocellulose.

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